Sample Preparation Methods

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A cryo high-vacuum shuttle for (correlative) cryogenic electron microscopy

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Preservation of the native state during preparation is a prerequisite for an unadulterated view on the specimen. Offering the possibility of the direct, unaltered portrayal of hydrated objects like organelles [1,2], viruses [3], bacteria [4,5] or eukaryotic cells [6], cryogenic techniques, e.g. cryo-electron microscopy (cryo-EM), became increasingly popular. The prerequisite of cryo-EM techniques is the embedding in vitreous (amorphous) ice [7]. Besides offering a snapshot of the pristine architecture of the specimen, the physical fixation method also accompanies with some challenging requirements for the subsequent handling: Maintenance of the temperature below the recrystallization temperature of -138°K [7] and the avoidance of any contamination, e.g. additional ice. Especially in the case of cryo-electron tomography (cryo-ET) [8,9], quantitative scanning transmission electron microscopy (q-STEM, [10,11]), or correlative cryo light and electron microscopy [12] the handling becomes demanding due to the complex postprocessing [13] or the highest requests to the purity of the specimen [14].
In the past, several concepts were introduced and they are now commercially available. Owing to miscellaneous disadvantages of the existing systems, either by not offering a high-vacuum environment or limiting the application to a restricted workflow, some improvements are still desirable. To the best of our knowledge, a cryo high-vacuum transfer system for (scanning) transmission electron microscopes (STEM and TEM accordingly), comparable to the EM VCT 100 (Leica Microsystems Inc., [15]) for scanning electron microscopy, has not yet been invented.
Here, we present a cryo high-vacuum system (CHVS) for cryogenic (correlative) experiments which streamlines the handling of frozen-hydrated specimen by considering the problems identified above. Figure 1 shows the main parts of the CHVS: the storage unit, the cryo high-vacuum shuttle and the “plug & play” docking device in combination with the cryo-holder (CT3500, Gatan Inc.) for the electron microscope.

However, once mounted to modified cryo-holder cartridges, up to eight standard EM grids can be transferred to the cooling stage of the storage unit (Figure 1a)). Afterwards, the cartridges can be transferred to the electron microscope or any other device which was extended by the docking device for the cryo high-vacuum shuttle. A constant vacuum level of $7\pm2 \times 10^{-5}$ Pa and a temperature of approximate 90 °K guarantee a contamination free transfer (Figure 2b). Finally, screwed to the pre-cooled cryo-holder, the entire transfer process of the capsules takes 5 minutes (Figure 1c)). In order to observe the transfer process to the cryo-holder inside the microscope chamber, a CCD camera was installed beside the docking device.

Providing the back and forth exchange of the cartridge, the cryo-holder can maintain at cryogenic temperatures throughout the day. Additionally, we believe that this system is the missing link for the correlation of different types of experiments, e.g. cryo-STEM, q-STEM, cryo-EM, cryo atomic force microscopy or cryo light microscopy. With this progress, we aim for better quantitative studies of organic and inorganic samples like proteinaceous specimen, DNA-protein complexes, thin films and nanoparticles as well as correlative studies of cells like neurons or subcellular components like synaptic vesicles [16].

1. S. Takamori et al, Cell 127 (2006), p. 831.
2. D. Nicastro et al, Journal of Structural Biology 129 (2000), p. 48.
3. S. Jun et al, Structure 19 (2011), p. 1573.
4. J. L. S. Milne and S. Subramaniam, Nature Reviews Microbiology 7 (2009), p. 666.
5. A. J. Koster et al, Journal of Structural Biology 120 (1997), p. 276.
6. O. Medalia et al, Science 298 (2002), p. 1209.
7. J. Dubochet et al, Quarterly Reviews of Biophysics 21 (1988), p. 129.
8. D. Vanhecke et al, Journal of Microscopy 242 (2011), p. 221.
9. R. Glaeser and R. Hall, Biophysical Journal 100 (2011), p. 2331.
10. J. S. Wall and J. F. Hainfeld, Annual Review of Biophysics and Biophysical Chemistry 15 (1986), p. 355.
11. A. A. Sousa and R. D. Leapman, Ultramicroscopy 123 (2012), p. 38.
12. J. Plitzko et al, Current Opinion in Biotechnology 20 (2009), p. 83.
13. A. Rigort et al, Journal of Structural Biology 172 (2010), p. 169.
14. S. Müller et al, Ultramicroscopy 46 (1992), p. 317.
15. R. Wepf et al, Microscopy and Microanalysis 10 (Suppl. 2) (2004), p. 970.
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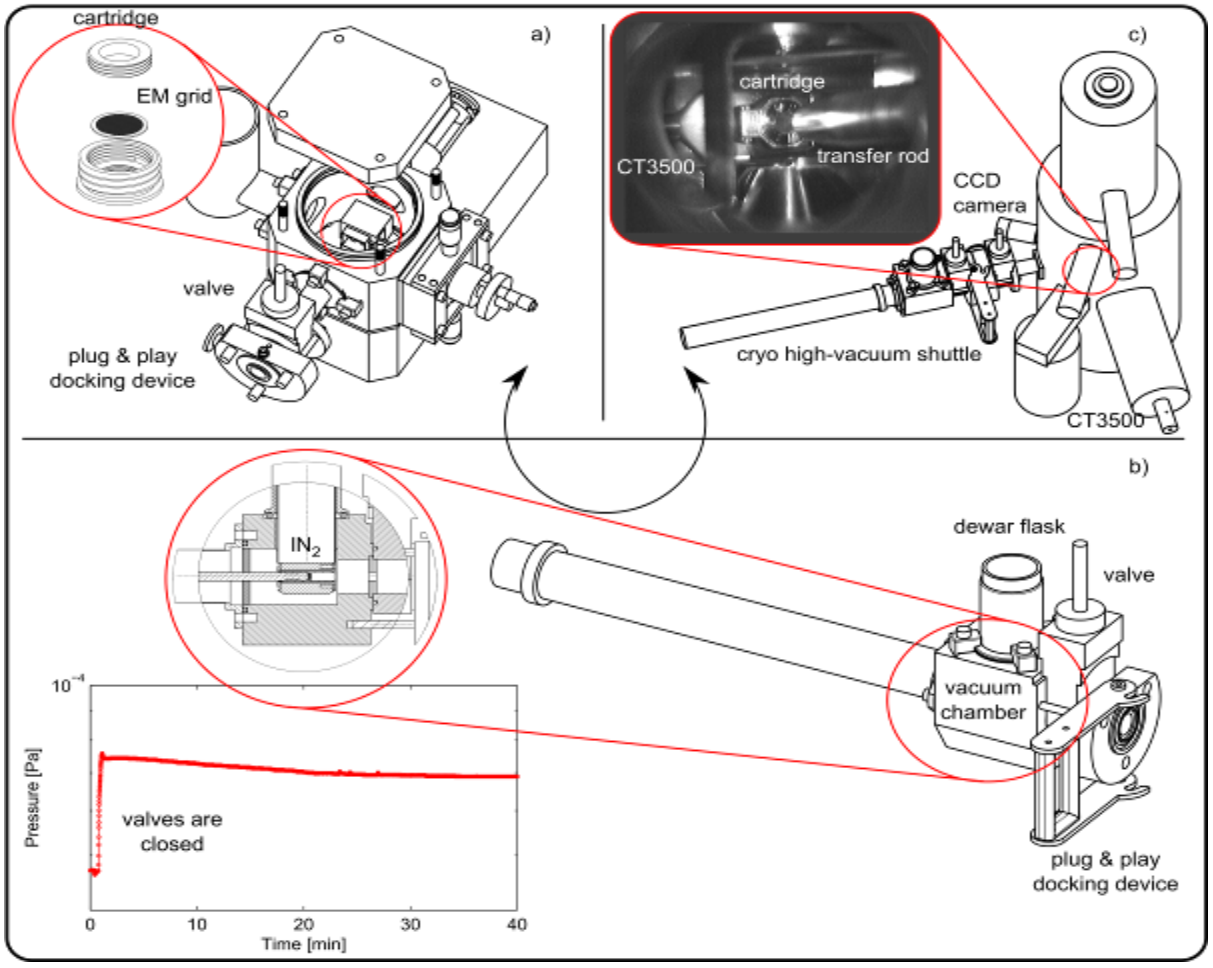

Figure 1. Schematic overview of the CHVS. a) Storage unit: Close up shows the modified cartridge which is finally mounted to the CT3500. Additionally, the small circle highlights the cooling stage. b) Cryo high-vacuum shuttle with general components: The top left close up shows the anti-contaminator of the cryo high-vacuum shuttle. On the left side: Vacuum level after disconnecting the shuttle from the docking device. c) Transfer of the capsule to the CT3500 inside the electron microscope (S-5000, Hitachi Ltd., Japan) visualized by a CCD camera